

Short communication

Gum and protein enrichment from sicklepod (*Cassia obtusifolia*) seed by fine grinding and sieving

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Abstract

Sicklepod (*Cassia obtusifolia*) is a weed species that has contaminated soybean and other crops. Although sicklepod continues to be a problem weed, the advent of herbicide-resistant soybeans has reduced the problem somewhat in the last few years. Sicklepod seed contains a gum of commercial interest in addition to protein and fat. An inexpensive process to enrich gum and protein contents of sicklepod seed is desirable for increased utilization and further processing. This study was conducted to determine whether fine grinding and sieving of sicklepod seed into separate fractions according to particle size could enrich gum and protein. Sicklepod seed was finely ground in a pin mill at different speed, and the resulting ground seed was separated into various fractions according to particle size. Gum content increased with particle size, and protein content decreased with particle size. Fine grinding and sieving of sicklepod seed can enrich gum in the fraction with largest particle size and can enrich protein in the fraction with smallest particle size. Fine grinding and sieving of sicklepod seed can make further processing more economical.

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Keywords: Sicklepod seed; Gum; Protein; Grinding; Sieving

1. Introduction

Sicklepod (*Cassia obtusifolia*) is a weed species that has contaminated soybean and other field crops. Although sicklepod continues to be a problem weed, the advent of herbicide-resistant soybeans and other crops has reduced the problem somewhat in the last few years. Sicklepod seed contain 14–19% protein,

5–6% fat, and 66–69% carbohydrate (Crawford et al., 1990). Abbott et al. (1998) extracted 41% of the sicklepod seed weight with 0.5 mol/l sodium hydroxide, and gum accounted for a large part of the extract. Fine grinding and air classification of ground seeds according to particle size can enrich gum (Wu and Abbott, 1996), protein, starch, fat, and sterol ferulates (Wu and Norton, 2001), and β -glucan (Wu and Doehlert, 2002). Seed gum can be used in food, feed, paper, textile, petroleum recovery, and pharmaceutical industries. The purpose of this study is to determine the effect of fine grinding and sieving on

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the enrichment of gum and protein in sicklepod seed fractions.

2. Materials and methods

Sicklepod (*C. obtusifolia*) seed was from North Carolina grown in 2000. The seeds were 4–5 mm long, angular, with several flattened surfaces, dark brown, surface waxy or glossy; yellow scars run on opposite surfaces along the long axis of the seed. The seed was ground once at 9000 rpm in an Alpine model 160Z pin mill (Augsbury, Germany) and separated into various fractions by sieving with screens with openings of 210, 420, 500, 710, and 1000 μm . In another experiment, the seed was ground three times at 14,000 rpm in the pin mill and fractionated in a Pillsbury laboratory model air classifier (Minneapolis, MN) with a cut point of 30 μm to obtain a fine and a coarse fraction. The coarse fraction was separated by sieving with screens with openings of 44, 53, 62, 74, 88, 105, 125, 149, 210, 297, and 500 μm .

Nitrogen, moisture and crude fat were determined by the Association of Official Analytical Chemists (AOAC) Methods 990.03, 934.01, and 920.39 (Horwitz, 2000). Nitrogen was from combustion analysis in a LECO CHN 2000 (Saint Joseph, MI) apparatus and crude fat was from petroleum ether extraction in a Tecator Soxtec System 1043 extraction unit (Foss AB, Hoganas, Sweden). Sicklepod fraction was hydrolyzed by 2 M/l trifluoroacetic acid to sugars, and sugars were converted to peracetylated aldononitrile derivatives. Standards made from galactose and mannose in various ratios were used to calibrate the analysis by gas chromatography (GC).

Details of GC sample preparation and analysis were described by Hou et al. (1996). For high performance liquid chromatography (HPLC) analysis of galactose and mannose, the trifluoroacetic acid hydrolyzate was filtered through a 0.45 μm syringe filter. The clarified solution was analyzed by an Aminex HPX-87H carbohydrate analysis column (Bio-Rad Laboratories, Hercules, CA) with 5 mmol/l sulfuric acid as eluant at 0.6 ml/min at 65 °C and an RI-150 refractive index detector (ThermoElectron, Schaumburg, IL). Gum content was corrected for the polymer repeat unit reduced molecular weight.

Anthraquinone was determined by measuring the absorbance at 520 nm of the alkaline extract after treatment with sodium hydroxide solution, using 1,8-dihydroxyanthraquinone as standard (Koshioka and Takino, 1978). The data were treated by analysis of variance, and Tukey's Studentized range test was used to determine significant difference at $P < 0.05$ (SAS Institute, Cary, NC).

3. Results and discussion

The yield and composition of pin milling sicklepod seed once at 9000 rpm are listed in Table 1. The >1000 μm fraction had high gum content and high mannose/galactose ratio but low protein and fat contents compared with the starting material. This >1000 μm fraction also accounted for 39% of the seed weight. The gum content and mannose/galactose ratio decreased as the particle size decreased. On the other hand, protein and fat contents increased as the particle size decreased.

Table 1
Yield and composition of 1 \times 9000 rpm pin-milled sicklepod

Size fraction (μm)	Yield (%)	Protein ($N \times 6.25$) (%)	Gum (%)	Mannose/galactose ratio	Fat (%)
>1000	39.1	5.9 ^a g	54.8 a	6.8 (0.6) ^b	0.2 e
710–1000	2.3	9.4 f	36.8 b	4.2	2.2 d
500–710	8.3	12.9 e	19.3 d	3.4	2.6 d
420–500	2.9	14.3 d	17.4 de	3.0	2.7 cd
210–420	28.7	23.4 b	10.6 ef	1.3	5.0 b
<210	18.7	26.4 a	9.7 f	0.7	8.2 a
1 \times 9000 rpm		15.7 c	29.2 c	3.6	3.4 c

^a Values followed by the same letter in a column are not significantly different ($P > 0.05$).

^b Values in parenthesis are standard deviation.

Table 2

Yield and composition of $3 \times 14,000$ rpm pin-milled sicklepod

Size fraction (μm)	Yield (%)	Protein ($N \times 6.25$) (%)	Gum (%)	Mannose/galactose ratio	Fat (%)
>500	27.4	5.5 ^a k	68.5 a	10.3	0.2 k
297–500	4.7	5.3 k	54.3 b		0.7 j
210–297	5.1	5.2 k	34.4 c		1.0 j
149–210	7.6	6.8 j	23.0 d	4.3	1.6 i
125–149	2.7	10.6 i	21.7 d		3.7 h
105–125	2.5	13.9 h	18.7 de		5.1 g
88–105	3.2	16.1 g	18.4 de	2.9	6.3 f
74–88	2.3	17.2 ef	19.0 de		6.4 f
62–74	3.4	17.4 e	17.3 de		7.0 e
53–62	3.5	19.3 d	14.1 def	1.7	7.6 d
44–53	2.5	21.5 c	14.2 def		8.9 c
30–44	1.6	26.5 b	10.0 ef		11.0 a
<30	33.5	29.6 a	7.0 f	0.4	9.5 b

^a Values followed by the same letter in a column are not significantly different ($P > 0.05$) from duplicate experiments.

Sicklepod gum is sold as DiagumTMCS and consists of polysaccharide chains with a 5:1 to 6:1 range of mannosyl to galactosyl units. Gas chromatography of sicklepod fractions showed small amounts of xylose, arabinose, glucose, and large amounts of mannose and galactose. The HPLC column used gave gum value as the sum of mannose and galactose as these eluted together. The ratio of mannose and galactose was obtained by GC. The HPLC column used required a minimum of sample preparation, but the GC method involved tedious sample preparation. Therefore, only a limited number of samples were determined by GC. Another HPLC method to determine mannose and galactose separately is under development.

Table 2 lists the yield and composition of pin milling sicklepod seed three times at 14,000 rpm. The >500 μm fraction accounted for 27% of the seed weight and had high gum content and high mannose/galactose ratio. The gum content and mannose/galactose ratio decreased as the particle size decreased. The protein and fat contents increased as the particle size decreased in general.

The effect of more intense grinding at $3 \times 14,000$ rpm compared with 1×9000 rpm was that a smaller percentage of seed weight with higher gum content and higher mannose/galactose ratio resulted for the fraction with largest particle size. Different mannose to galactose ratios for guar and locust bean gums have different solubilities and viscosity properties. The fractions with different mannose/galactose ratios may be used for a variety of applications.

The anthraquinone content of sicklepod ranged from 0.4% for $3 \times 14,000$ rpm pin-milled >500 μm fraction to 1.3% for <30 μm fraction in Table 2 (not shown). For food and feed use these values must be reduced. Bayerlein et al. (1989) used a mixture of water and alkanol (such as isopropanol or ethanol) or a mixture of water and acetone to produce colorless, odorless, and tasteless cassia endosperm flour. A similar procedure can be used to reduce the anthraquinone content in our sicklepod fractions.

4. Conclusion

Fine grinding and sieving of sicklepod seed can enrich the gum in the coarse fraction and the protein in the fine fraction. Selective combination of intensity of grinding and subsequent sieving can make further processing more economical for the component(s) of interest.

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